CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date appearing below.

ELI LILLY AND COMPANY

By Pamela A Pettigrew Date July 12, 2004

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	James Arthur Hoffmann and Jirong Lu	)	
Serial No.	:	09/744,431	)	Group Art Unit:
Filed	:	January 22, 2001	)	1647
For	:	FSH and FSH VARIANT FORMULATIONS, PRODUCTS, AND METHODS	)	Examiner: R. DeBerry
Docket No.	:	X-12383M	)	

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michael R. DeFelippis, hereby state and declare that:

Background

1. I hold the degree of Doctor of Philosophy in Biochemistry from the Ohio State University, and I am currently a Research Advisor at Eli Lilly and Company (Lilly), Indianapolis, Indiana. I have been a scientist with Lilly since 1990 and have been involved with formulation development for numerous protein products. I have authored or co-authored numerous peer-reviewed publications, book chapters, and review articles. I am an inventor or co-inventor on seven U.S. Patents. My *curriculum vitae* is attached.

2. I have reviewed the above referenced U.S. Patent Application Serial No. 09/744,431 ("'431 Application") and the current claims. I have also reviewed the most recent Office Action for the '431 Application, mailed on March 11, 2004, as well as the references cited in the Office Action. These references include EP 0 853 945 by Skrabanja *et al.* ("Skrabanja"), U.S. Patent No. 6,267,958 by Andya *et al.* ("Andya"), and Keene *et al.* ("Keene"), *J. Biol. Chem.* 264(9):4769-75 (1989). I also reviewed the prior Office Actions and the references cited therein.
3. I understand that the '431 Application has an earliest effective filing date of July 23, 1998. This declaration describes the state of the art as I understood it while working as a formulation scientist on and before that date.
4. On May 18, 2004, I filed a declaration ("May 18 declaration") in a related case, U.S. Patent Application Serial No. 09/928,198 ("'198 Application"). I have attached a copy of the May 18 Declaration for convenience (*see* Attachment A). In the present declaration, I will make reference to the May 18 declaration.

The Invention

5. The '431 Application is directed to a liquid formulation of follicle stimulating hormone (FSH) that is suitable as a multi-dose product. Such a multi-dose product as described in the '431 Application: 1) must meet the requirements for anti-microbial effectiveness (*i.e.*, microbiological stability) during administration of the product, and 2) must be otherwise suitably stable prior to and during use (*i.e.*, chemical, physical, and conformational stability).
6. I understand that the amended claims of the '431 Application are directed to human FSH and a preservative in an aqueous diluent, wherein (a) the preservative is selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, and mixtures thereof, (b) the concentration of FSH is 5.0 μg/mL to 2 mg/mL, (c) the FSH consists of an α-subunit having SEQ ID NO:5 and a β-subunit having SEQ ID NO:6, held together by noncovalent interactions, and (d) the formulation is suitable for multi-dose administration by injection.
7. Thus, the claimed formulation requires a preservative: phenol, m-cresol, p-cresol, o-cresol, or mixtures thereof. Each of these is recognized in the art as a "preservative"

providing microbiological stability. I understand that a new FSH product, GONAL-F® RFF PEN, has recently been approved by the Food and Drug Administration (reference CCD). This product comprises human FSH and meta-cresol in an aqueous diluent as described by the claim.

Conformational and Physical Stability of Protein Formulations

8. As noted in the May 18 Declaration, the addition of a preservative to some protein formulations has been shown to have a destabilizing effect on physical properties of the protein. For examples of the destabilizing effect of preservatives on protein formulations, *see* Maa and Hsu, *Intl. J. Pharm.* 140:155-58 (1996), reference CBU on Applicants' Information Disclosure Form 1449; Lam *et al.*, *Pharm. Res.* 14(6):725-29 (1997), reference CAC; Remmele *et al.*, *Pharm. Res.* 15(2):200-08 (1998), reference CAA; Fransson *et al.*, *Pharm. Res.* 14(5):606-12 (1997), reference CAB; and Akers, *J. Pharm. Sci.* 91(11):2283-2300 (2002), reference CBW.
9. Yet, comparing the data provided in the '431 Application for the preserved liquid FSH formulations, the FSH heterodimer exhibits acceptable physical and conformational stability when compared to an unpreserved control FSH solution that lacks preservative. In my opinion, a person skilled in the art could not predict these results, nor would such a person have any expectations that such results could be achieved at the time of the effective filing date of the '431 Application.
10. Both physical and conformational stability are concerns for protein formulations. *See* May 18 Declaration, paragraphs 8 and 9.
11. Highly purified FSH was known to be conformationally unstable. (*See*, *inter alia*, Skrabanja, page 3, lines 51-54). This is especially true for relatively dilute aqueous solutions of FSH. *Id.* Because of concern regarding this instability, FSH was stored as a lyophilized powder and reconstituted with solvent immediately before use.
12. The effect of preservatives on the physical and/or conformational stability of FSH, when stored as a solution with benzyl alcohol, was not predictable from results achieved with other proteins. *See* May 18 Declaration, paragraphs 11 and 12. The same is true for FSH stored as a solution with a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, and mixtures thereof; neither the physical or conformational stability is predictable from results achieved with other proteins.

13. As stated in the May 18 Declaration, "In my opinion, an experienced formulator of therapeutic protein formulations would recognize that protein-preservative compatibility is dependent on the properties of the protein and preservative chosen. Therefore, compatibility is unpredictable without extensive experimentation. One cannot predict that any particular protein-preservative combination would be compatible, and thus lead to a stable formulation, merely because another protein demonstrated compatibility with that preservative." The addition of preservatives to pharmaceutical formulations is **not "routine,"** contrary to the statement on page 7 of the Office Action.
14. Moreover, FSH had known conformational instability (*see* Strickland and Puett, *Biol. Chem.* 257:2954-60 (1982), reference CAR; Reichert and Ramsey, *J. Biol. Chem.* 250:3034-40 (1975), reference CB); and FSH-containing products had long been sold as lyophilized preparations to be reconstituted, used immediately, and the remainder discarded. "Thus, having been sold in such a form for many years, it would have been reasonable to infer (particularly in view of the scientific literature and the heterodimer structure) that preserved, solution formulations were not stable. Accordingly, prior to the present invention, the data and circumstances suggested FSH would be unstable with preservatives." *See* May 18 Declaration, paragraph 14.

The Art Cited by the Examiner

15. The cited art references for the current application are the same references cited for the '198 Application and addressed in paragraphs 16 through 21 of the May 18 Declaration. Those references, collectively, did not make the physical and conformational stability of the claimed formulation in the '198 Application, comprising FSH and benzyl alcohol, predictable or provide any expectation of success. Nor do those references collectively provide predictability or expectation of success for the claimed formulation of the '431 Application, comprising FSH and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, and mixtures thereof.
16. Keene does not teach or suggest any formulation containing FSH.
17. Skrabanja does not teach or suggest that a preservative could or should be added to the FSH-containing composition.
18. Furthermore, Skrabanja describes the required and optional excipients of its formulation in detail. As noted in the May 18 Declaration, paragraphs 17 and 18, if Skrabanja

intended the use of additional excipients, such as a preservative, it certainly would have mentioned so. "I find it implausible that Skrabanja is silent on preservatives, given the known issues of preservative compatibility, the known instability of the FSH heterodimer, and the criticality of preservatives to any multi-dose preserved formulation. Thus, one skilled in the art is unable to draw any conclusions from these passages." *See* May 18 Declaration, paragraph 18.

19. Andya's extensive lists of possible proteins, including FSH, and possible excipients, including preservatives such as phenol, meta-cresol, and benzyl alcohol, do not suggest to one skilled in the art to use such a preservative with all of the proteins listed. As is true for benzyl alcohol, several of the proteins listed were known to be incompatible with phenol and meta-cresol. (*See* paragraph 8, *supra*). Accordingly, this reference does not convey that the entire list of proteins could be stably formulated with preservatives. Andya provides no expectation of success for a liquid FSH formulation preserved with phenol, m-cresol, p-cresol, o-cresol, or mixtures thereof.
20. Additionally, in my opinion, stability of Andya's extremely high concentrated protein solution does not predict or suggest stability of an FSH solution with a much lower concentration.
21. Thus, the cited art references collectively do not convey, suggest or motivate an ordinarily skilled formulation scientist to select phenol, m-cresol, p-cresol, o-cresol, or mixtures thereof, from the list of excipients in the lyophilized formulation of Andya, and to add such to the liquid gonadotropin formulation of Skrabanja, having a human FSH sequence as taught by Keene, with any expectation of success that such a combination would yield a preserved FSH formulation with the physical and conformational stability shown in the '431 application.

Summary

22. In summary, physical and conformational stability of a liquid formulation of FSH in a preserved aqueous solution is **not predictable from** the cited references or **the general state of the art**. The stability of a non-covalently bonded FSH heterodimer in a preserved liquid formulation was not known in the art at the time of the effective filing date, nor did anything in the art suggest that FSH would remain stable in such a formulation. The data and circumstances surrounding FSH in 1998 would suggest that

FSH may be unstable in the presence of a preservative. Thus, an FSH formulation preserved with a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, and mixtures thereof, and having the conformational and physical stability demonstrated in the '431 Application was unpredictable and unexpected.

23. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Michael R. DeFelippis, Ph.D.

July 12, 2004

Michael R. DeFelippis

488 Sapphire Drive
Carmel, IN 46032

Work: (317) 276-6027
Home: (317) 846-5561

Education

B.S. 1985: Fairleigh Dickinson University, Teaneck, NJ
Major: Biochemistry

Ph.D. 1990: The Ohio State University, Columbus, OH
Major: Biochemistry
Academic Advisor: Michael H. Klapper, Ph.D.
Thesis: The Redox Potentials of the Tyrosine and Tryptophan Radicals and Long-Range Electron Transfer Between Tyrosine and Tryptophan in Peptides

Employment/Research Background

Research Advisor, Biopharmaceutical Product Research and Development, 2004-present: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Senior Research Scientist, Pharmaceutical Product Development, 2000-2004: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Research Scientist, Biopharmaceutical Product Development, 1996-1999: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Senior Pharmaceutical Chemist, Biopharmaceutical Product Development, 1990-1995: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Research Associate and Teaching Assistant, 1985-1990: The Ohio State University, Columbus, Ohio

Visiting Graduate Student Researcher, December, 1986-January, 1987: Ben Gurion University, Beer Sheva, Israel

Undergraduate Researcher, Summer 1985: Savannah River Ecology Laboratory, Aiken, South Carolina

Honors/Awards

President's Award, Lilly Research Laboratories (1994).

2500 Day Speed to Market Award (1994), Eli Lilly and Company Product Development.

First place, Albert L. Henne Research Competition, Department of Chemistry, The Ohio State University (1989).

Dean's Award, College of Science and Engineering, Fairleigh Dickinson University, 1985.

Professional Affiliations

American Chemical Society, 1985-present

Peer Reviewed Publications

Snavely, W.K., Subramaniam, B., Rajewski, R.A. and DeFelippis, M.R. (2002) "Micronization of Insulin from Halogenated Alcohol Solution Using Supercritical Carbon Dioxide as an Antisolvent". J. Pharm. Sci. 91, 2026-2039.

Yip, C.M., Brader, M.L., Frank, B.H., DeFelippis, M.R., and Ward, M.D. (2000) "Structural Studies of a Crystalline Insulin Analog Complex with Protamine by Atomic Force Microscopy", *Biophys. J.* 78, 466-473.

Richards, J.P., Stickelmeyer, M.P., Frank, B.H., Pye, S., Barbeau, M., Radziuk, J., Smith, G.D., and DeFelippis, M.R. (1999) "Preparation of a Microcrystalline Suspension Formulation of Lys^{B28},Pro^{B29}-Human Insulin with Ultralente Properties", *J. Pharm. Sci.* 88, 861-867.

Beavis, R.C., Knierman, M.D., Sharknas, D.A., Heady, M.A., Frank, B.H., and DeFelippis, M.R. (1999) "A Novel Protein Cross-linking Reaction in Stressed Neutral Protamine Hagedorn Formulations of Insulin", *J. Pharm. Sci.* 88, 331-336.

Yip, C.M., DeFelippis, M.R., Frank, B.H., Brader, M.L., and Ward, M.D. (1998) "Structural and Morphological Characterization of Ultralente Insulin Crystals by Atomic Force Microscopy: Evidence of Hydrophobically Driven Assembly", *Biophys. J.* 75, 1172-1179.

Richards, J.P., Stickelmeyer, M.P., Flora, D.B., Chance, R.E., Frank B.H., and DeFelippis, M.R. (1998) "Self-Association Properties of Monomeric Insulin Analogs under Formulation Conditions", *Pharm. Res.* 15, 1434-1441.

Yip, C.M., Brader, M.L., DeFelippis, M.R., and Ward, M.D. (1998) "Atomic Force Microscopy of Crystalline Insulins: The Influence of Sequence Variation on Crystallization and Interfacial Structure". *Biophys. J.* 74, 2199-2209.

DeFelippis, M.R., Bakaysa, D.L., Bell, M.A., Heady, M.A., Li., S., Youngman, K.M., Pi, S., Radziuk, J., and Frank., B.H. (1998) "Preparation and Characterization of a Cocrystalline Suspension of [Lys^{B28}, Pro^{B29}]-Human Insulin Analog", *J. Pharm. Sci.* 87, 170-176.

Birnbaum D.T. , Kilcomons M.A., DeFelippis M.R., and Beals, J.M. (1997) "Assembly and Dissociation of Human Insulin and $Lys^{B28}Pro^{B29}$-Insulin Hexamers: A Comparison Study", *Pharm. Res.* 14, 25-36.

Pikard, R.T., Chiou, X.G., Strifler, B.A., DeFelippis, M.R., Hyslop, P.A., Tebbe, A.L., Yee, Y.K., Reynolds, L.J., Dennis, E.A., Kramer, R.M., and Sharp, J.D. (1996) "Indentification of Essential Residues for the Catalytic Function of 85-kDa Cytosolic Phospholipase A_2: Probing the Role of Histidine, Aspartic Acid, Cysteine and Arginine", *J. Biol. Chem.* 271, 19225-19231.

Youngman, K.M., Spencer, D.B., Brems, D.N., and DeFelippis, M.R. (1995) "Kinetic Analysis of the Folding of Human Growth Hormone: Influence of Disulfide Bonds", *J. Biol. Chem.* 270, 19816-19822.

DeFelippis, M.R., Kilcomons, M.A., Lents, M.P., Youngman, K.M., and Havel, H.A. (1995) "Acid Stabilization of Human Growth Hormone Equilibrium Folding Intermediates", *Biochim. Biophys. Acta.* 1247, 35-45.

DeFelippis, M.R., Alter, L.A., Pekar, A.H., Havel, H.A., and Brems, D.N. (1993) "Evidence for a Self-Associating Equilibrium Intermediate during Folding of Human Growth Hormone", *Biochemistry* 32, 1555-1562.

Klapper, M.H., DeFelippis, M.R., Lee, H., Mishra, A.K., and Faraggi, M. (1991) "Effects of Structure on Long Range Electron Transfer in Peptides", in The Proceedings of the 9th International Congress of Radiation Research (Eds., Dewey, W. C., Edington, M, Fry, R. J. M., Hall, E. J., and Whitmore, G. F.) *Radiation Research* Volume 2.

Lee, H., DeFelippis, M.R., Faraggi, M., and Klapper, M.H. (1991) "Long Range Electron Transfer (LRET) in Proteins: Implications for Oxidative Damage", in the *Proceedings 5th Meeting International Society Free Radical Research.*

Weinstein, M., Alfassi, Z.B., DeFelippis, M.R., Klapper, M.H., and Faraggi, M. (1991) "Long Range Electron Transfer Between Tyrosine and Tryptophan in Hen Egg-White Lysozyme", *Biochim. Biophys. Acta*, 1076, 173-178.

DeFelippis, M.R., Murthy, C.P., Broitman, F., Weinraub, D., Faraggi, M., and Klapper, M.H. (1991) "Electrochemical Properties of Tyrosine Phenoxy and Tryptophan Indolyl Radicals in Peptides and Amino Acid Analogues", *J. Phys. Chem.*, 95, 3416-3419.

DeFelippis, M.R., Faraggi, M., and Klapper, M.H. (1990) "Evidence for Through-Bond Long Range Electron Transfer in Peptides", *J. Amer. Chem. Soc.*, 112, 5640-5642.

DeFelippis, M.R., Faraggi, M., and Klapper, M.H. (1989) "Redox Potentials of the Azide and Dithiocyanate Radicals", *J. Phys. Chem.*, 94, 2420-2424.

Faraggi, M., DeFelippis, M.R., and Klapper, M.H. (1989) "Long Range Electron Transfer between Tyrosine and Tryptophan in Peptides", *J. Amer. Chem. Soc.*, 111, 5141-5145.

DeFelippis, M.R., Murthy, C.P., Faraggi, M., and Klapper, M.H. (1989) "Pulse Radiolytic Measurement of Redox Potentials: The Tyrosine and Tryptophan Radicals", *Biochemistry*, 28, 4847-4853.

Faraggi, M., Weinraub, D., Broitman, F., DeFelippis, M.R., and Klapper, M.H. (1988) "One Electron Oxidations of Ferrocenes: A Pulse Radiolysis Study", *Radiat. Phys. Chem.*, 32, 293-297.

Invited Book Chapters and Review Articles

DeFelippis, M.R. (2003) "Overcoming the Challenges of Noninvasive Protein and Peptide Delivery". *Am. Pharmaceut. Rev.* 6, 21-30.

DeFelippis, M.R., Chance, R.E., and Frank, B.H. (2003) "Insulin Chemistry and Pharmacokinetics" in *Ellenberg & Rifkin's Diabetes Mellitus*, 6th Edition, Porte, Jr., D., Sherwin, R.S. and Baron, A. (Eds.) McGraw-Hill, New York, Chapter 28.

Beals, J.M., Brader, M.L., DeFelippis, M.R., and Kovach, P.M. (2002) "Insulin" in *Pharmaceutical Biotechnology An Introduction for Pharmacists and Pharmaceutical Scientists*, 2nd Edition, Daan J. A. Crommelin, D.J.A. and Sindelar, R.D. (Eds.) Taylor & Francis Limited, London, Chapter 10.

DeFelippis, M.R., Chance, R.E., and Frank, B.H. (2001) "Insulin Self-Association and the Relationship to Pharmacokinetics and Pharmacodynamics", *Critical Reviews in Therapeutic Drug Carrier Systems*, 18(2), 201-264.

DeFelippis, M.R. and Akers, M. (2000) "Peptides and Proteins as Parenteral Suspensions: an Overview of Design, Development, and Manufacturing Considerations", in *Pharmaceutical Formulation Development of Peptides and Proteins*, Frokjaer, S. and Hovgaard, L. (Eds.) Taylor & Francis Limited, London, 113-144.

Akers, M and DeFelippis, M.R. (2000) "Peptides and Proteins as Parenteral Solutions", in *Pharmaceutical Formulation Development of Peptides and Proteins*, Frokjaer, S. and Hovgaard, L. (Eds.) Taylor & Francis Limited, London, 145-177.

Presentations and Meeting Abstracts

DeFelippis, M.R. (2003) "Comparability Assessment: Role of Formulation". Invited oral presentation at the PDA/IABS Conference on Comparability. Prague, Czech Republic, February 28.

DeFelippis, M.R. (2003) "Overcoming the Challenges of Noninvasive Protein and Preptide Delivery". Invited oral presentation at CBI's 6th Drug Delivery Conference, Philadelphia, PA, April 8.

DeFelippis, M.R. (2003) "Comparability Assessment: Role of Formulation". Invited oral presentation at the Health Canada Shared Learning Symposium. Toronto, Canada, November 18.

Khan, A. and DeFelippis, M.R. (2002) "How Technological Advances in Protein and Peptide Delivery Systems Ensure Commercial Success". Oral Presentation at CBI's 5th Drug Delivery Conference, Philadelphia, PA, April 8-9.

Dobbins, M.A., DeFelippis, M.R., Frank, B.H. and VanAntwerp, W. (2002) "Solution Formulation of Insulin Lispro with Increased Physical Stability for Pump Application". Poster presented at Formulation Strategies for Biopharmaceuticals Conference, Miami, FL, February 4-6.

DeFelippis, M.R. (2001) "Relating the Physical Properties of Insulin to Pharmacology", Invited Lecture, University of Kansas, April 25.
Snavely, W.K., Subramaniam, B., Rajewski, R.A., and DeFelippis, M.R. 2000. "Micronization of Insulin from Organic Solution Using Supercritical Carbon Dioxide as Antisolvent". Poster presented at the AAPS Meeting, Indianapolis, IN, October 29-November 2.

K.S. Looney, M.R. DeFelippis, J.D. Hofer & B.H. Frank. 1998. "The chemical stability of insulin lispro protamine suspension and insulin lispro mixtures". Poster presented at the 34th Annual Meeting of the EASD, Barcelona , Spain, September 8-12.

C.A. Siedlecki, M.L. Brader, M.D. Ward, M. Tirrell & M.R. DeFelippis. 1998. "Insulin fibrils observed by atomic force microscopy". Lecture presented at the 216th ACS National Meeting and Exposition, Boston, MA, August 23-27.

M.A. Heady, D.A. Sharknas, M.R. DeFelippis, B.H. Frank, M.D. Knierman & R. C. Beavis. 1998. "Characterization of high molecular mass proteins in insulin-protamine suspensions". Poster presented at the 12th Symposium of the Protein Society, San Diego, CA July 25-29.

S. Li, J.M. Beals, S.W. Dodd, J.D. Hofer & M.R. DeFelippis. 1998. "Adsorption of [LysB28,ProB29]-human insulin (lyspro) onto lyspro-protamine crystals". Poster presented at the 12th Symposium of the Protein Society, San Diego, CA July 25-29.

J.P. Richards, M.P. Stickelmeyer, S. Pye, M. Barbeau, J. Radziuk, B.H. Frank & M.R. DeFelippis. 1998. "Preparation and Characterization of a Microcrystalline Suspension Formulation of LysB28ProB29-Human Insulin", published abstract *Diabetes*, 47 (Supp. 1): A352.

J.P. Richards, M.R. DeFelippis, D.B. Flora, R.E. Chance, B.H. Frank B.H. & M.P. Stickelmeyer, M.P. 1997. "Association Properties of Monomeric Insulin Analogs Under Formulation Conditions". Poster Presented at the First Annual Beckman Symposium on Solution Interaction of Macromolecules, Galveston, Texas, November 14-17.

C.M. Yip, M.R. DeFelippis, M.R. Ward & M.L. Brader. 1997. "In Situ Determination of Molecular Packing and Growth Mechanisms in Protein Crystals: Atomic Force Microscopy of Insulin and Insulin Analogs". Lecture Presented at the American Crystallographic Association Annual Meeting, St. Louis, Missouri, July 19–25.

M.R. DeFelippis, C.M. Yip, M.D. Ward & M.L. Brader. 1997. "Structural Studies of an Insulin Analog Cocrystal Form by Atomic Force Microscopy". Lecture Presented at the American Crystallographic Association Annual Meeting, St. Louis, Missouri, July 19–25.

E. Ciszak, B.H. Frank, J.M. Beals, C.M. Yip, M.R. DeFelippis & G.D. Smith. 1997. "Structural Studies of Lispro-Insulins". Poster Presented at the American Crystallographic Association Annual Meeting, St. Louis, Missouri, July 19–25.

C.M. Yip, M.D. Ward, M.L. Brader & M.R. DeFelippis. 1997. "Atomic Force Microscopy of a Monomeric Insulin Analog Crystal Form: Comparison with Native Structure". Poster presented at the 41st Annual Meeting of the Biophysical Society, New Orleans, Louisiana, March 2–6.

M.R. DeFelippis. 1996. "Pharmaceutical Research and Development". Invited Lecture for a course on Clinical Drug Development: A Regulatory Review. Butler University, September 11th.

J.R. Radziuk, B. Bradley, L. Welsh, M.R. DeFelippis & P. Roach. 1996. "Neutral Protamine Lispro: Activity Profile of S.C. Administration with and without Admixture of Soluble Lispro". 32nd Annual Meeting of the European Association for the Study of Diabetes, Vienna, Austria, 1–5 September

M.R. DeFelippis, D.L. Bakaysa, K.M. Youngman, J. Radziuk & B.H. Frank. 1996. "Preparation and characterization of neutral protamine lispro (NPL) suspension". Diabetes 45 Suppl 2:74A. Abstract 267.

J. Radziuk, B. Bradley, L. Welsh, M.R. DeFelippis & P. Roach. 1996. "Profiles of biological activity after subcutaneous administration of mixtures of LysB28-ProB29 human insulin (lispro) in soluble and neutral protamine formulations". Diabetes 45 Suppl 2:218A. Abstract 800.

D.L. Bakaysa, B.H. Frank, K.M. Youngman & M.R. DeFelippis. 1996. "Biphasic Mixture Formulations of a Rapid-Acting Insulin Analog" poster presented at the 211th ACS National Meeting held in New Orleans, March 24-28.

S.L. Edwards, M.R. DeFelippis, B.H. Frank, M.A. Kilcomons, T. A. Sheliga, M.P. Stickelmeyer, K.M. Youngman & H.A. Havel. 1996. "Assessment of the Stability of Insulin Lispro Mixtures with Human Insulin NPH" poster presented at the 211th ACS National Meeting held in New Orleans, March 24-28.

K.M. Youngman, D.L. Bakaysa, M.A. Kilcomons & M.R. DeFelippis. 1996. "Crystallization of Lys^{B28}-Pro^{B29} Human Insulin to Extend Formulation Applications" poster presented at the 211th ACS National Meeting held in New Orleans, March 24-28.

Pickard, R.T., Chiou, X.G., Strifler, B.A., DeFelippis, M.R., Hyslop, P.A., Tebbe, A.L., Yee, Y.K., Kramer, R.M. & Sharp, J.D. 1995. "Probing the Catalytic Center of Cytosolic Phospholipase A2 with Mutagenesis". Poster presented at the FASEB Summer Conference on Phospholipases, July.

Birnbaum, D.T., Kilcomons, M.A., Beals, J. M. & DeFelippis, M.R., "Formation and Disruption Kinetics of Cobalt-Insulin Hexamers: Ligand/Anion Binding and Cooperativity". Poster presented at the 9th Symposium of The Protein Society, July 1995.

Youngman, K.M., Spencer, D.B., Brems, D.N & DeFelippis, M.R., "Folding Kinetics of Human Growth Hormone". Poster presented at the 8th Symposium of The Protein Society, San Diego, California, July 1994.

Spencer, D.B., Youngman, K.M., DeFelippis, M.R. & Brems, D.N., "Folding Kinetics of a Cysteine-Modified Form of Human Growth Hormone". Poster presented at the 8th Symposium of The Protein Society, San Diego, California, July 1994.

DeFelippis, M.R., Kilcomons, M.A., Lents, M.P., Youngman, K.M. & Havel, H.A., "Acid Conformation of Human Growth Hormone Provides New Insight into the Equilibrium Folding Mechanism". Poster presented at the 38th Annual Meeting of the Biophysical Society, New Orleans, Louisiana, March 1994.

DeFelippis, M.R., Alter, L.A., Pekar, A.H., Havel, H.A. & Brems, D.N., "Equilibrium Folding Pathway of Human Growth Hormone Contains a Self-Associating Intermediate". Poster presented at the 6th Symposium of The Protein Society, San Diego, California, July 1992.

Havel, H.A., Millican, R.L. & DeFelippis, M.R., "Investigations to Determine the Molecular Mechanism of Human Insulin Aggregation". Poster presented at the 6th Symposium of The Protein Society, San Diego, California, July 1992.

Klapper, M.H., DeFelippis, M.R., Lee, H. & Faraggi, M., "Effects of Structure on Long Range Electron Transfer in Peptides". Presented at the 9th International Congress of Radiation Research, Toronto, Canada, July 1991.

DeFelippis, M.R., Faraggi, M. & Klapper, M.H., "Redox Potentials of the Tyrosine and Tryptophan Radicals". Poster presented at the XVI Midwest Enzyme Conference, Evanston, Illinois, October, 1989.

DeFelippis, M.R., Faraggi, M. & Klapper, M.H., "Long Range Electron Transfer (LRET) Between Tyrosine and Tryptophan in Peptides". Poster presented at the XVI Midwest Enzyme Conference, Evanston, Illinois, October, 1989.

DeFelippis, M.R., Faraggi, M. & Klapper, M.H., "Long Range Electron Transfer in Proteins and Polypeptides. The Construction of Molecular 'Wires'". Poster presented at the 33rd Annual Meeting of the Biophysical Society, Cincinnati, Ohio, February, 1989.

Patents

DeFelippis, M.R., Dobbins, M.A., Frank, B.H., Li, S. & Rebhun, D.M., U.S. Patent number 6,551,992.

DeFelippis, M.R., Dobbins, M.A., Frank, B.H., Li, S. & Rebhun, D.M., U.S. Patent number 6,034,054.

DeFelippis, M.R. and Frank, B.H., U.S. Patent number 5,952,297.

DeFelippis, M.R., U.S. Patent number 5,747,642.

DeFelippis, M.R., U.S. Patent number 5,650,486.

Anderson, J.H., Jr., DeFelippis, M.R., Frank, B.H. & Havel, H.A., U.S. Patent number 5,547,929.

DeFelippis, M.R., U.S. Patent number 5,461,031.

ATTACHMENT A: Declaration of May 18, 2004 by Dr. DeFelippis

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	James Arthur Hoffmann and Jirong Lu	)
Serial No.	:	09/928,198	)
Filed	:	August 10, 2001	) Group Art Unit: 1647
For	:	FSH FORMULATION	) Examiner: R. DeBerry
Docket No.	:	X-12383N	)

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michael R. DeFelippis, hereby state and declare that:

Background

1. I hold the degree of Doctor of Philosophy in Biochemistry from the Ohio State University, and I am currently a Research Advisor at Eli Lilly and Company (Lilly), Indianapolis, Indiana. I have been a scientist with Lilly since 1990 and have been involved with formulation development for numerous protein products. I have authored or co-authored numerous peer-reviewed publications, book chapters, and review articles. I am an inventor or co-inventor on seven U.S. Patents. My *curriculum vitae* is attached.
2. I have read and understand the above referenced U.S. Patent Application Serial No. 09/928,198 ("'198 Application") and the current claims. I have also read and understand the most recent Office Action for the '198 Application, mailed on March 18, 2004, as well as the references cited in the Office Action. These references include EP 0 853 945 by Skrabanja *et al.* ("Skrabanja"), U.S. Patent No. 6,267,958 by Andya *et al.* ("Andya"),

and Keene *et al.* ("Keene"), *J. Biol. Chem.* 264(9):4769-75 (1989). I also reviewed the prior Office Actions, the references cited therein, and the Declarations filed therewith.

3. I understand that the '198 Application has an earliest effective filing date of July 23, 1998. This declaration describes the state of the art as I understood it while working as a formulation scientist on and before that date.

The Invention

4. The '198 Application is directed to a liquid formulation of follicle stimulating hormone (FSH) that is suitable as a multi-dose product. To be suitable as a multi-dose product, the formulation: 1) must meet the requirements for anti-microbial effectiveness (*i.e.*, microbiological stability), and 2) must be otherwise suitably stable prior to and during use (*i.e.*, chemical, physical, and conformational stability). I understand that the pending claims of the '198 Application are directed to human FSH and benzyl alcohol in an aqueous diluent, wherein (a) the concentration of FSH is 5.0 μg/mL to 2 mg/mL, (b) the FSH consists of an α-subunit having SEQ ID NO:5 and a β-subunit having SEQ ID NO:6, held together by noncovalent interactions, and (c) the formulation is suitable for multi-dose administration by injection.

5. The claimed formulation requires benzyl alcohol. Benzyl alcohol is recognized in the art as a "preservative." That is, it provides microbiological stability. I understand that two products are now commercially sold, PUREGON® brand FSH and GONAL-F® Multi-Dose brand FSH. Both products comprise human FSH and benzyl alcohol in an aqueous diluent as described by the claim.

Conformational and Physical Stability of Protein Formulations

6. The addition of a preservative, including benzyl alcohol, to some protein formulations has been shown to have a destabilizing effect on physical properties of the protein. For example, meta-cresol and benzyl alcohol are known to cause aggregation of some single-chain protein molecules. See, for example, Maa and Hsu, *Intl. J. Pharm.* 140:155-58 (1996), reference CBU on Applicants' Information Disclosure Form 1449; Lam *et al.*, *Pharm. Res.* 14(6):725-29 (1997); Remmele *et al.*, *Pharm. Res.* 15(2):200-08 (1998), reference CAC; Fransson *et al.*, *Pharm. Res.* 14(5):606-12 (1997), reference CAB; and Akers, *J. Pharm. Sci.* 91(11):2283-2300 (2002), reference CCB.

7. Yet, in the preserved liquid formulation of the '198 Application, the FSH heterodimer exhibits comparable physical and conformational stability to an unpreserved control FSH solution that lacks benzyl alcohol. In my opinion, a person skilled in the art could not predict these results, nor would such a person have any expectations that such results could be achieved at the time of the effective filing date of the '198 Application.
8. For protein formulations, physical stability of the protein is one concern. Physical stability is a measure of undesirable aggregation of the protein: typically a decreased propensity towards aggregation is correlated with greater physical stability of the protein.
9. Another concern is conformational stability. FSH is a non-covalently bonded glycoprotein heterodimer comprised of an α-subunit and a ß-subunit. The quaternary structure of the heterodimer (*i.e.,* subunit association) results from weak interactions between the subunits known as non-covalent bonds. These include hydrophobic and electrostatic interactions, hydrogen bonding, and van der Waals forces. Because the interactions are relatively weak, non-covalent bonds are more easily disrupted than covalent bonds. For example, the non-covalent bonds between the α- and ß-subunits of FSH are weaker than the crosslinking covalent bonds between the A and B chains of insulin. If the quaternary structure of FSH is disrupted and the subunits dissociate, FSH becomes biologically inactive. Thus, FSH must be formulated such that the subunits maintain association. The ability to maintain the heterodimer structure is referred to as conformational stability.
10. Highly purified FSH was known to be conformationally unstable. (See, *inter alia,* Skrabanja, page 3, lines 51-54). This is especially true for relatively dilute aqueous solutions of FSH. *Id.* Because of this instability, FSH was stored as a lyophilized powder and reconstituted with solvent immediately before use.
11. The conformational stability of FSH, a non-covalently bonded heterodimer, when stored as a solution with a preservative such as benzyl alcohol, was not predictable from results achieved with other proteins.
12. Moreover, the effect of preservatives on the physical stability of FSH when stored in a solution with a preservative such as benzyl alcohol was not predictable from results achieved with other proteins.

13. In my opinion, an experienced formulator of therapeutic protein formulations would recognize that protein-preservative compatibility is dependent on the properties of the protein and preservative chosen. Therefore, compatibility is unpredictable without extensive experimentation. One cannot predict that any particular protein-preservative combination would be compatible, and thus lead to a stable formulation, merely because another protein demonstrated compatibility with that preservative. For example, although some proteins (*e.g.*, human chorionic gonadotropin and erythropoietin) have been shown to be stable over a one or two month period using benzyl alcohol as a preservative, others (e.g., interferon-γ, interleukin 1-R, and insulin-like growth factor 1) have been demonstrated to be physically unstable when preserved with benzyl alcohol (Lam *et al.*, *Pharm. Res.* 14(6):725-29 (1997); Remmele *et al.*, *Pharm. Res.* 15(2):200-08 (1998); and Fransson *et al.*, *Pharm. Res.* 14(5):606-12 (1997), respectively).
14. While conformational stability and physical stability cannot be predicted from the successes or failures of other proteins, the evidence available to an ordinarily skilled artisan at the time of the effective filing date of the '198 Application suggested that FSH would be unstable with many excipients, including benzyl alcohol. For example, FSH had known conformational instability (see Strickland and Puett, *Biol. Chem.* 257:2954-60 (1982), reference CAR; Reichert and Ramsey, *J. Biol. Chem.* 250:3034-40 (1975), reference CB); and products comprising FSH had a long history of being sold as lyophilized preparations to be reconstituted, used immediately, and the remainder discarded. From a formulation scientist's perspective, lyophilized single-dose vials would only be used for highly unstable proteins. Thus, having been sold in such a form for many years, it would have been reasonable to infer (particularly in view of the scientific literature and the heterodimer structure) that preserved, solution formulations were not stable. Accordingly, prior to the present invention, the data and circumstances suggested FSH would be unstable with preservatives, including benzyl alcohol.

The Art Cited by the Examiner

15. The cited art references collectively do not make the physical and conformational stability of the claimed formulation predictable, nor do the references provide any expectation of success.

16. Keene merely teaches the construction of vectors and the expression of human FSH using these vectors. This reference does not teach or even suggest any formulation containing FSH, especially not an FSH formulation preserved with benzyl alcohol.
17. Skrabanja teaches that a liquid formulation of FSH and citrate can be stabilized by the addition of a sufficient amount of a thioether, preferably methionine, to the formulation. This reference is quite complete, describing the required and optional excipients in detail. Skrabanja does not convey that a stable liquid formulation could be achieved by other means. Nor does it suggest that a preservative such as benzyl alcohol could or should be added to the FSH-containing composition.
18. Skrabanja makes reference to the Becton-Dickinson B-D pen injector. I am familiar with this device. Skrabanja also notes that "the liquid medicament can be in the form of a cartridge for multiple use." These passages are unclear. If Skrabanja intended these passages to imply the use of additional excipients such as a preservative, I find it implausible that Skrabanja is silent on preservatives, given the known issues of preservative compatibility, the known instability of the FSH heterodimer, and the criticality of preservatives to any multi-dose preserved formulation. Thus, one skilled in the art is unable to draw any conclusions from these passages.
19. Andya teaches a lyophilized protein formulation which can be reconstituted to generate a high concentration solution suitable for injection. FSH is one of an extensive list of possible proteins for use in such formulation. Benzyl alcohol is included in a similarly extensive list of possible excipients and is identified as a preferred preservative. However, in my opinion, one skilled in the art would not interpret this as a suggestion to use benzyl alcohol with all of the proteins listed. Several of the proteins listed were known to be incompatible with benzyl alcohol. (See paragraph 12, *supra*). Accordingly, this reference simply conveys to the ordinary skilled formulation scientist that benzyl alcohol would be a preferred preservative for those antibodies described and exemplified, anti-HER2 and anti-IgE. This reference does not convey that the entire list of proteins could be stably formulated with benzyl alcohol.
20. Given that hundreds of proteins are listed in Andya (FSH is one in the list), a formulation scientist would understand that stability of the formulation would depend upon which protein, preservative, and other excipients were chosen, and that the stability would need

to be determined through testing. Andya does not convey that all formulations containing a protein and a preservative would be pharmaceutically acceptable and stable.

21. Moreover, Andya's invention requires a very high protein concentration (≥50 mg protein/mL diluent), whereas the claimed invention of the '198 Application requires a concentration of 5.0 μg/mL to 2 mg/mL. Thus, the protein concentrations in Andya are at least 25 times more concentrated, and perhaps even 10,000 times or more concentrated. As mentioned in paragraph 9 of this declaration, at the time of the effective filing date, relatively dilute solutions of FSH were known to be conformationally unstable (Skrabanja, page 2, 42-45). In my opinion, stability of Andya's extremely high concentrated protein solution does not predict or suggest stability of an FSH solution with a much lower concentration.
22. Thus, the cited art references collectively do not convey, suggest or motivate an ordinarily skilled formulation scientist to select benzyl alcohol from the list of excipients in the lyophilized formulation of Andya, and to add such to the liquid gonadotropin formulation of Skrabanja, having a human FSH sequence as taught by Keene, with any expectation of success that such a combination would yield a preserved FSH formulation that is as stable as an FSH formulation that does not contain such preservative.

Summary

23. In summary, physical and conformational stability of a liquid formulation of FSH in a preserved aqueous solution is not predictable from the cited references or the general state of the art. The stability of a non-covalently bonded FSH heterodimer in a preserved liquid formulation was not known in the art at the time of the effective filing date, nor did anything in the art suggest that FSH would remain stable in such a formulation. The data and circumstances surrounding FSH in 1998 would suggest that FSH may be unstable in the presence of a preservative and benzyl alcohol in particular. Thus, the stable formulation achieved in the '198 Application was unpredictable and unexpected.

24. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

_____

Michael R. DeFelippis, Ph.D.